Longitudinal CSF biomarkers in patients with early Parkinson disease and healthy controls

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Brit Mollenhauer, MD
Chelsea J. Caspell-Garcia, MS
Christopher S. Coffey, PhD
Peggy Taylor, ScD
Leslie M. Shaw, PhD
John Q. Trojanowski, MD, PhD
Andy Singleton, PhD
Mark Frasier, PhD
Kenneth Marek, MD
Douglas Galasko, MD
For the Parkinson's Progression Marker Initiative

Correspondence to Dr. Mollenhauer: brit.mollenhauer@med.uni-goettingen.de

ABSTRACT

Objective: To analyze longitudinal levels of CSF biomarkers in drug-naive patients with Parkinson disease (PD) and healthy controls (HC), examine the extent to which these biomarker changes relate to clinical measures of PD, and identify what may influence them.

Methods: CSF α-synuclein (α-syn), total and phosphorylated tau (t- and p-tau), and β-amyloid 1–42 (Aβ42) were measured at baseline and 6 and 12 months in 173 patients with PD and 112 matched HC in the international multicenter Parkinson's Progression Marker Initiative. Baseline clinical and demographic variables, PD medications, neuroimaging, and genetic variables were evaluated as potential predictors of CSF biomarker changes.

Results: CSF biomarkers were stable over 6 and 12 months, and there was a small but significant increase in CSF Aβ42 in both patients with patients with PD and HC from baseline to 12 months. The t-tau remained stable. The p-tau increased marginally more in patients with PD than in HC. α-syn remained relatively stable in patients with PD and HC. Ratios of p-tau/t-tau increased, while t-tau/Aβ42 decreased over 12 months in patients with PD. CSF biomarker changes did not correlate with changes in Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale motor scores or dopamine imaging. CSF α-syn levels at 12 months were lower in patients with PD treated with dopamine replacement therapy, especially dopamine agonists.

Conclusions: These core CSF biomarkers remained stable over 6 and 12 months in patients with early PD and HC. PD medication use may influence CSF α-syn. Novel biomarkers are needed to better profile progressive neurodegeneration in PD. ***Neurology*® 2017;89:1959–1969**

GLOSSARY

α-syn = α-synuclein; **Aβ42** = β-amyloid 1-42; **AD** = Alzheimer disease; **HC** = healthy controls; **LED** = levodopa equivalent dose; **MDS-UPDRS** = Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale; **MoCA** = Montreal Cognitive Assessment; **p-tau** = phosphorylated tau protein; **PD** = Parkinson disease; **Penn** = University of Pennsylvania; **PPMI** = Parkinson's Progression Biomarker Initiative; **RBD** = REM sleep behavior disorder; **RBD-SQ** = REM sleep behavior disorder screening questionnaire; **t-tau** = total tau protein.

Intracellular accumulation of α-synuclein (α-syn) aggregates, neuronal dysfunction and loss, and synaptic changes are the neuropathologic hallmarks of Parkinson disease (PD). Mutations and duplications in the α-syn encoding gene (*SNCA*) are associated with autosomal dominantly inherited PD, providing further support for a central role of α-syn in PD.[1] Recent evidence suggests that transcellular spread of aggregated or misfolded α-syn may contribute to progression[2] via the extracellular space. This raises the possibility that the quantification of α-syn in extracellular fluids may be a marker for PD diagnosis and progression. Total tau (t-tau) and phosphorylated tau (p-tau)

Supplemental data at Neurology.org

From the Department of Neurology (B.M.), University Medical Center, Göttingen; Paracelsus-Elena-Klinik (B.M.), Kassel, Germany; Department of Biostatistics (C.J.C.-G., C.S.C.), College of Public Health, University of Iowa, Iowa City; BioLegend Inc. (P.T.), San Diego, CA; Department of Pathology & Laboratory Medicine (L.M.S., J.Q.T.), Center for Neurodegenerative Disease Research, Institute on Aging (L.M.S. , J.Q.T.), and Morris K. Udall Center of Excellence for Parkinson's Disease Research (J.Q.T.), Perelman School of Medicine, University of Pennsylvania, Philadelphia; Molecular Genetics Section (A.S.), Laboratory of Neurogenetics, National Institute on Aging, NIH, Bethesda, MD; The Michael J. Fox Foundation for Parkinson's Research (M.F.), New York, NY; Institute for Neurodegenerative Disorders (K.M.), New Haven, CT; and Department of Neurosciences (D.G.), University of California, San Diego.

Coinvestigators are listed at Neurology.org.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article. The Article Processing Charge was funded by The Michael J. Fox Foundation for Parkinson's Research.

protein, as well as β-amyloid 1-42 (Aβ42), correlate with key pathologic features in Alzheimer disease (AD).[3,4] These proteins have been shown to be relevant in PD neurodegeneration with an association between the microtubule-associated protein tau (*MAPT*) gene with PD and a known overlapping pathology (with AD). In cross-sectional studies, levels of α-syn in CSF are decreased in PD and related disorders.[5–7] This decrease of CSF α-syn and changes in t-tau and p-tau protein and Aβ42 was recently replicated in the large multicenter Parkinson's Progression Biomarker Initiative (PPMI).[8,9] Longitudinal changes in levels of CSF α-syn and other biomarkers in PD were examined in other cohorts,[10–14] with suggestions that CSF α-syn may increase over time or in those with more severe PD. Understanding the dynamics of changes in biomarkers may advance our understanding of the pathobiology of the disease course, identify contributions of different pathologies to progression,[15,16] and can provide benchmark data for the design and interpretation of disease-modifying clinical trials that use biomarkers for participant enrollment or as outcome measures.

We therefore analyzed the levels of α-syn, tau, p-tau, and Aβ42 in CSF samples of patients with PD and healthy controls (HC) at baseline and 6- and 12-month follow-up in the PPMI cohort. We hypothesized that these core CSF biomarkers would be stable in patients with PD and HC and would correlate with clinical or ^{123}I-ioflupane dopamine transporter imaging (DaTscan) indices of disease progression.

METHODS

Participants. People with recently diagnosed untreated PD were enrolled in PPMI. PPMI is an ongoing prospective longitudinal, observational, international multicenter study that aims to identify biomarkers for the progression of PD. As described previously,[9] newly diagnosed, drug-naive patients with PD (n = 423), age- and sex-matched HC (n = 196), and participants with scans without evidence of dopaminergic deficit syndrome (n = 60) were included in the study. Recruitment took place between June 2010 and May 2013, in 21 PD centers in the United States and Europe in accordance with PPMI protocols (ppmi-info.org/study-design).[17] The criteria for enrollment between June 2010 and May 2013 for participants with PD were (1) age over 30 years; (2) presence of 2 of the following: bradykinesia, rigidity, and resting tremor, or presence of an asymmetric resting tremor, or asymmetric bradykinesia; (3) diagnosis recently made within the last 24 months; (4) PD drug naivety; and (5) dopamine transporter deficit in the putamen on the DaTscan by central reading. This article is based on the data from CSF samples obtained at baseline and 6- and 12-month follow-up visits and analyzed for t-tau and p-tau, Aβ42, and α-syn. Our findings reflect data collected as of January 19, 2016, from the PPMI database (ppmi-info.org).

Standard protocol approvals, registrations, and patient consents. Approval was received from the ethical standards committee on human experimentation for all experiments with human participants. Written informed consent was obtained from all study participants (consent for research). The study is registered in clinicaltrials.gov as NCT01141023.

CSF sample collection and analysis. CSF was collected using standardized lumbar puncture procedures. Sample handling, shipment, and storage were carried out as described in our previous study[8] and the PPMI biologics manual (ppmi-info.org). Aliquots of 0.5 mL frozen CSF were shipped from the PPMI Biorepository Core laboratories to the University of Pennsylvania (Penn) PPMI Biomarker Core and to BioLegend (San Diego, CA) for analyses. Measurements of CSF Aβ42, tau, and p-tau were made using the xMAP-Luminex platform with INNOBIA AlzBio3 immunoassay kit–based reagents (Fujirebio-Innogenetics, Ghent, Belgium) at Penn, as we have described elsewhere.[8] Commercially available sandwich type immunoassay kits (BioLegend; formerly Covance) were used to analyze CSF α-syn and CSF hemoglobin levels, as described previously.[8]

Clinical assessment measures. The clinical assessment battery is described on the PPMI website. In brief, motor assessment was performed with the Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS)[18] III and total score. At baseline, all participants with PD were free of dopamine-related medications. Use of medications for PD was recorded at the 6- and 12-month visits, and is expressed as levodopa equivalent doses (LEDs).[19]

Cognitive testing comprised screening with the Montreal Cognitive Assessment (MoCA) and the Hopkins Verbal Learning Test–revised, processing speed/attention was assessed using the Symbol Digit Modality Test, executive function/working memory was assessed with the Wechsler Memory Scale III Letter-Number Sequencing Test, and visuospatial abilities were assessed with the Benton Judgment of Line Orientation test. The REM sleep behavior disorder (RBD) screening questionnaire (RBD-SQ) was used to assess RBD.[20]

Dopamine SPECT imaging. Dopamine imaging was performed by DaTscan using standardized methods, as described.[17] We analyzed whether quantitative DaTscan measures of caudate, putamen, or striatal uptake were related to CSF biomarker changes.

Genetic variables. To examine whether selected genetic variants were associated with CSF biomarkers, we used data for *APOE* genotypes and single nucleotide polymorphisms related to *SNCA*. These were measured by the PPMI Genetics Core as previously described.[17,21]

Statistical analysis. All analyses are based on data retrieved from the PPMI website, when all biomarkers for the 6- and 12-month follow-up periods were available on January 19, 2016. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). All tests performed using the CSF biomarkers were rank-based. *t* Tests or χ^2 were used to compare baseline demographic and clinical variables in participants with longitudinal CSF data vs participants who only had baseline CSF data; these comparisons were performed separately in patients with PD and controls. Nonparametric tests were used where specified in the tables. Repeated-measures linear mixed models were used to test for changes in CSF biomarker levels from

Table 1 Baseline clinical characteristics of patients with Parkinson disease (PD) and healthy controls (HC) with longitudinal CSF data

	Patients with PD (n = 173)	HC (n = 112)
Age at baseline lumbar puncture, y		
n	173	112
Mean (SD)	60.91 (9.3)	60.02 (11.8)
95% CI	59.5–62.3	57.8–62.2
Sex, n (%)		
Men	124 (72)	70 (63)
Women	49 (28)	42 (38)
Age at PD onset, y		
n	169	NA
Mean (SD)	58.79 (9.7)	NA
95% CI	57.3–60.3	NA
MDS-UPDRS part III (motor) score		
n	173	112
Mean (SD)	21.86 (8.6)	1.38 (2.4)
95% CI	20.6–23.2	0.9–1.8
MDS-UPDRS total score		
n	173	112
Mean (SD)	33.61 (13.4)	4.54 (4.3)
95% CI	31.6–35.6	3.7–5.3
TD/non-TD classification, n (%)		
TD	126 (73)	NA
Non-TD	47 (27)	NA
HVLT total recall		
n	173	112
Mean (SD)	24.43 (4.5)	26.18 (4.5)
95% CI	23.8–25.1	25.3–27.0
HVLT delayed recall		
n	173	112
Mean (SD)	8.29 (2.4)	9.41 (2.3)
95% CI	7.9–8.7	9.0–9.8
HVLT discrimination recognition		
n	173	112
Mean (SD)	9.62 (2.5)	9.87 (3.4)
95% CI	9.3–10.0	9.2–10.5
MoCA		
n	173	112
Mean (SD)	27.04 (2.2)	28.25 (1.1)
95% CI	26.7–27.4	28.0–28.5
SDMT		
n	173	112
Mean (SD)	41.43 (8.8)	46.54 (11.3)
95% CI	40.1–42.8	44.4–48.7
LNS		
n	173	112

Continued

baseline to 6 and 12 months. In cases where the change in CSF biomarker levels was significant at the 0.1 level, simple linear models were used to analyze potential baseline predictors of change. First, the univariate relationship between each predictor and the biomarker change was examined. Then, any variables that had univariate associations with p values less than 0.2 were included in a multivariable model. Finally, a backwards selection process was used to remove variables individually until all variables remaining in the model were significant at the 0.01 level.

Repeated-measures linear mixed models were also used to test for overall differences in CSF levels, MDS-UPDRS, and DaTscan levels between groups over time. In each of these models, an interaction between time and group was tested first, before testing for an overall group difference. Tests of interactions were reported where p values were significant at the 0.1 level. If the test of interaction was not significant, the interaction term was removed from the model and a test for overall group difference was reported.

Spearman rank correlation coefficients between changes in CSF biomarker levels and changes in MDS-UPDRS scores and DaTscan variables were also reported. In addition, repeated-measures linear mixed models were used to examine longitudinal relationships between CSF biomarker levels and DaTscan levels and PD medication use.

Unless otherwise specified, a significance level of $p = 0.01$ was used as the cutoff to account for multiple comparisons. A more formal method of adjustment for multiple comparisons was not used, as the authors believed this would have been too stringent given the exploratory nature of these analyses.

RESULTS Demographic and clinical data of the 173 patients and 112 controls are shown in table 1. The flow of participants is shown in figure e-1 at Neurology.org and associated genetic and imaging data in these cohorts in table e-1. Comparisons between this longitudinal CSF cohort and the remaining 239 PPMI patients and 77 controls (with baseline CSF data only) revealed small differences in sex (more men were in the longitudinal CSF data group; $p = 0.03$). Also, baseline CSF Aβ42 and p-tau values were slightly lower in the longitudinal cohort compared to those with only baseline data available ($p = 0.05$ and 0.01, respectively) (table e-2).

Changes in CSF biomarkers in patients with PD and controls over time are shown in table 2. Levels of Aβ42 increased slightly in patients with PD and HC, which was significant from baseline to 12 months. While t-tau remained relatively stable over time in both groups, p-tau significantly increased in patients with PD (baseline vs 12 months) but not in controls (table 2). The ratio of p-tau to t-tau increased between baseline and 12 months and the ratio of t-tau to Aβ42 declined slightly over time in the patients with PD only. CSF total α-syn levels remained relatively stable, as did the ratio of t-tau to α-syn from baseline to 12 months (table 2).

Comparing changes in CSF biomarkers over time in the PD group with those in controls, we found that t-tau, p-tau, and α-syn levels in patients with PD were significantly lower than in HC across time points. When samples with hemoglobin in CSF >200 ng/mL were excluded (PD n = 91 and HC n = 69) the comparison of patients with PD vs HC

Table 1 Continued

	Patients with PD (n = 173)	HC (n = 112)
Mean (SD)	10.84 (2.3)	10.86 (2.6)
95% CI	10.5–11.2	10.4–11.3
BJLO		
n	173	112
Mean (SD)	12.94 (2.1)	13.21 (1.9)
95% CI	12.6–13.3	12.9–13.6

Abbreviations: BJLO = Benton Judgment of Line Orientation test; CI = confidence interval; HVLT = Hopkins Verbal Learning Test; LNS = Letter-Number Sequencing Test; MDS-UPDRS = Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale; MoCA = Montreal Cognitive Assessment; NA = not applicable; SDMT = Symbol Digit Modality Test; TD = tremor dominant.

became nonsignificant, probably due to fewer participants (table 3).

In multivariate regression analysis that examined baseline predictors of changes in biomarkers, we found that no cognitive test was a significant predictor of changes in levels of CSF biomarkers. Similarly, cognitive dysfunction (MoCA <26), the presence of hallucinations (MDS-UPDRS I.2 ≥1), and the presence of RBD (RBD-SQ ≥5) in the patients with PD at baseline were not significant predictors of any of the CSF biomarkers. A limited number of genetic variants were examined as predictors, including *APOE* ε4 and polymorphisms in the *SNCA* gene. Among these, the genetic polymorphism at *SNCA* rs3910105 was a significant predictor for the change in Aβ42. The increase of p-tau and the ratios p-tau/t-tau and p-tau/Aβ42 were significantly predicted by sex (men having a more pronounced increase) and by the polymorphism of *SNCA* rs 356181. The decrease of t-tau/Aβ42 was significantly predicted by the polymorphism of SNCA rs3910105 (data not shown). Table e-3 shows predictors of significant changes in Aβ42 and p-tau from baseline to 12 months in patients with PD.

We also examined correlations between changes in CSF biomarkers and key clinical measures (MDS-UPDRS part III score and total score), each of which increased over 6 and 12 months in PD (tables 4 and 5). However, after adjustment for multiple comparisons, no significant correlations were observed.

We analyzed whether the use and LED of PD medications during the 12 months follow-up period were associated with changes in CSF biomarkers (table 5). We found that patients using PD medications had greater decreases in α-syn than those who did not take medications. This was driven by the subgroup who used dopamine agonists, not other dopamine replacement (tables e-4 and e-5), and there was only a weak relationship with LED. There was no relationship with changes in other CSF biomarkers.

There were moderate and significant correlations among the 4 CSF markers, which are summarized in table e-6.

DISCUSSION This multicenter longitudinal study evaluated core CSF biomarkers—including α-syn, Aβ42, t-tau, and p-tau levels—measured over 6 and 12 months in patients with de novo PD and healthy controls. The strengths of the data include quality control and standardization of recruitment of participants, support of clinical diagnosis through DaTscan imaging, rigorous clinical assessment, CSF and biosample collection, handling and central analysis according to established standardized operational procedures, and functional as well as structural brain image analysis, together with high recruitment numbers, retention rates, and rates of performance of longitudinal lumbar punctures. The diversity of enrollment sites is representative of a typical multicenter interventional study. Therefore, these data can serve as a benchmark for future intervention trials.

Overall, we show stability of all 4 biomarkers during 12 months of follow-up in de novo PD. Therefore, these CSF biomarkers do not mirror disease progression, in particular progressive striatonigral degeneration as evaluated by clinical motor ratings (MDS-UPDRS III) and DaTscan measures. Whether these CSF biomarkers change over a longer time course, during more advanced stages of PD, or in relation to, for example, blood–brain barrier changes, can be reassessed once further PPMI biomarker analyses are conducted.

CSF α-syn assays measure the total physiologic protein rather than its select pathologic forms, and cellular events that lead to its release into extracellular CSF are not well-understood. The development of assays that measure other forms of α-syn such as Pser129 α-syn[22] or α-syn oligomers[23,24] may provide stronger indices of disease activity. Although it is possible that decreased levels of CSF α-syn in PD may normalize (increase) with effective neuroprotective therapy as target engagement, this will need to be tested in the setting of an effective intervention. CSF levels of t-tau and p-tau181 levels have been extensively studied in AD, where they are related to neuronal damage and neurofibrillary changes. They increase in the presymptomatic mild cognitive impairment stage, and remain stably elevated or even decrease slightly once the symptomatic phase with memory loss is present.[25,26] It may therefore be important to similarly analyze people at risk for PD, such as asymptomatic mutation carriers and people with idiopathic nonmotor symptoms, such as RBD or hyposmia.[27]

Analyses of t-tau and p-tau proteins and Aβ42 in longitudinal CSF samples in 403 drug-naive patients

Table 2 Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS), DaTscan levels, and CSF analytes (β-amyloid 1-42 [Aβ42], total tau protein [t-tau], phosphorylated tau protein [p-tau], α-synuclein [α-syn], and their ratios) at baseline and 6 and 12 months in patients with Parkinson disease (PD) and healthy controls (HC)

	Patients with PD					HC				
Variable	Baseline	Change at 6 months	Change at 12 months	*p* Value (baseline vs 6 months)	*p* Value (baseline vs 12 months)	Baseline	Change at 6 months	Change at 12 months	*p* Value (baseline vs 6 months)	*p* Value (baseline vs 12 months)
MDS-UPDRS III				<0.0001	<0.0001				NA	0.007
n	173	173	148			112	NA	112		
Mean (SD)	21.86 (8.6)	3.41 (6.0)	4.46 (7.9)			1.38 (2.4)		0.53 (2.0)		
Min, max	20.6, 23.2	2.5, 4.3	3.2, 5.7			0.9, 1.8		0.1, 0.9		
Mean caudate				NA	<0.0001				NA	NA
n	172	NA	169			112	NA	NA		
Mean (SD)	1.99 (0.549)		−0.22 (0.27)			2.98 (0.63)				
Min, max	1.90, 2.07		−0.26, −0.18			2.86, 3.09				
Mean putamen				NA	<0.0001				NA	NA
n	172	NA	169			112	NA	NA		
Mean (SD)	0.79 (0.28)		−0.11 (0.15)			2.09 (0.55)				
Min, max	0.75, 0.84		−0.14, −0.10			1.99, 2.20				
Mean striatum				NA	<0.0001				NA	NA
n	172	NA	169			112	NA	NA		
Mean (SD)	1.39 (0.39)		−0.17 (0.19)			2.53 (0.57)				
Min, max	1.33, 1.45		−0.20, −0.14			2.43, 2.64				
Aβ42				0.33	0.01				0.09	0.002
n	173	173	173			112	112	112		
Median	361.10	7.50	11.50			378.15	6.15	21.35		
Min, max	139.9, 670.0	−242.2, 205.0	−207.1, 316.8			88.8, 680.3	−230.3, 152.4	−265.3, 190.0		
t-tau				0.10	0.56				0.81	0.30
n	171	171	170			110	110	110		
Median	38.70	−1.20	−0.15			44.65	−0.25	0.70		
Min, max	15.6, 121.0	−15.4, 21.8	−28.4, 36.7			18.4, 188.2	−26.6, 35.4	−27.8, 39.6		
p-tau				0.32	0.001				0.19	0.15
n	173	173	172			112	112	112		
Median	11.40	0.20	1.95			14.00	−0.35	0.55		
Min, max	4.7, 39.7	−21.7, 40.3	−26.5, 46.6			6.1, 58.5	−24.3, 39.3	−30.4, 74.6		

Continued

Table 2 Continued

	Patients with PD					HC				
Variable	Baseline	Change at 6 months	Change at 12 months	*p* Value (baseline vs 6 months)	*p* Value (baseline vs 12 months)	Baseline	Change at 6 months	Change at 12 months	*p* Value (baseline vs 6 months)	*p* Value (baseline vs 12 months)
α-syn				0.43	0.79				0.24	0.96
n	173	172	173			112	112	112		
Median	1714.39	31.95	14.15			1950.13	8.81	−5.00		
Min, max	332.93, 6694.55	−4503.15, 1301.97	−4602.02, 1540.90			592.56, 5237.68	−1912.52, 2258.43	−2292.36, 1748.10		
α-syn[a]				0.84	0.99				0.77	0.55
n	82	81	82			43	43	43		
Median	1712.71	33.74	55.01			1941.14	5.26	−101.64		
Min, max	581.17, 5110.77	−2239.79, 837.42	−2107.86, 1284.25			683.94, 5237.68	−1912.52, 1164.75	−2292.36, 1529.98		
p-tau/t-tau				0.34	0.002				0.39	0.43
n	171	171	169			110	110	110		
Median	0.297	0.014	0.044			0.29	−0.01	0.02		
Min, max	0.150, 0.883	−0.659, 1.006	−0.607, 2.127			0.13, 0.79	−0.51, 0.44	−0.54, 1.37		
t-tau/Aβ42				0.007	0.003				0.80	0.57
n	171	171	170			110	110	110		
Median	0.11	−0.01	−0.01			0.111	−0.002	−0.003		
Min, max	0.06, 0.53	−0.06, 0.24	−0.15, 0.27			0.071, 2.12	−0.278, 0.08	−0.199, 0.15		
p-tau/Aβ42				0.42	0.01				0.04	0.73
n	173	173	172			112	112	112		
Median	0.03	0.00	0.01			0.04	−0.00	0.00		
Min, max	0.01, 0.17	−0.07, 0.09	−0.08, 0.23			0.02, 0.66	−0.23, 0.07	−0.19, 0.39		
t-tau/α-syn				0.09	0.61				0.32	0.47
n	171	170	170			110	110	110		
Median	0.02	−0.001	−0.001			0.02	−0.001	0.00		
Min, max	0.01, 0.07	−0.05, 0.02	−0.04, 0.02			0.01, 0.06	−0.03, 0.02	−0.03, 0.02		
t-tau/α-syn[a]				0.12	0.21				0.38	0.70
n	82	81	81			43	43	43		
Median	0.02	−0.00	−0.00			0.024	−0.001	0.000		
Min, max	0.01, 0.05	−0.03, 0.02	−0.02, 0.02			0.01, 0.06	−0.03, 0.01	−0.03, 0.02		

Abbreviation: NA = not applicable.

p Values are based on the ranks of the biological variables. DaTscan is not performed at 6 months in patients with PD, and is performed only at baseline in HC.

[a] Subset of participants with hemoglobin <200 ng/mL at all time points, excluding those with missing hemoglobin values at one or more time points.

Table 3 Comparison over time in patients with Parkinson disease (PD) vs healthy controls (HC) of CSF levels of β-amyloid 1-42 (Aβ42), total tau protein (t-tau), phosphorylated tau protein (p-tau), and α-synuclein (α-syn)

	Patients with PD			HC			
Variables	Baseline	6 months	12 months	Baseline	6 months	12 months	*p* Value (PD vs HC)
Aβ42							0.134
n	173	173	173	112	112	112	
Median	361.10	361.70	375.50	378.15	373.80	396.80	
Min, max	139.90, 670.00	129.30, 687.00	144.10, 732.50	88.80, 680.30	98.00, 609.80	95.20, 691.30	
t-tau							0.0004
n	171	173	172	110	112	112	
Median	38.70	37.40	39.05	44.65	44.75	45.15	
Min, max	15.60, 121.00	15.60, 134.70	16.60, 128.80	18.40, 188.20	16.80, 180.50	19.40, 216.20	
p-tau							0.006
n	173	173	172	112	112	112	
Median	11.40	11.50	14.10	14.00	13.30	15.75	
Min, max	4.70, 39.70	5.10, 56.30	5.40, 61.80	6.10, 58.50	6.00, 52.50	6.00, 89.50	
α-syn							0.002
n	173	172	173	112	112	112	
Median	1714.39	1779.73	1720.77	1950.13	2069.87	2036.88	
Min, max	332.93, 6694.55	472.92, 4659.05	352.36, 5157.08	592.56, 5237.68	658.61, 5208.86	729.32, 5295.43	
α-syn[a]							0.109
n	82	81	82	43	43	43	
Median	1712.71	1802.91	1816.15	1941.14	1981.53	2018.74	
Min, max	581.17, 5110.77	707.06, 4264.73	797.87, 5157.08	683.94, 5237.68	890.57, 5157.39	729.32, 4784.16	
p-tau/t-tau							0.215
n	171	173	171	110	112	112	
Median	0.30	0.29	0.340	0.29	0.27	0.31	
Min, max	0.15, 0.88	0.13, 1.29	0.07, 2.48	0.13, 0.79	0.14, 0.95	0.14, 1.58	
t-tau/Aβ42							0.002
n	171	173	172	110	112	112	
Median	0.11	0.10	0.10	0.111	0.115	0.112	
Min, max	0.06, 0.53	0.06, 0.48	0.06, 0.51	0.071, 2.12	0.07, 1.84	0.06, 2.27	
p-tau/Aβ42							0.085
n	173	173	172	112	112	112	
Median	0.03	0.03	0.04	0.04	0.03	0.04	
Min, max	0.01, 0.17	0.01, 0.14	0.01, 0.28	0.02, 0.66	0.02, 0.43	0.02, 0.50	
t-tau/α-syn							0.202
n	171	172	172	110	112	112	
Median	0.02	0.02	0.02	0.02	0.02	0.02	
Min, max	0.01, 0.07	0.01, 0.05	0.01, 0.06	0.01, 0.06	0.01, 0.05	0.01, 0.05	
t-tau/α-syn[a]							0.515
n	82	81	81	43	43	43	
Median	0.02	0.02	0.02	0.02	0.02	0.02	
Min, max	0.01, 0.05	0.01, 0.05	0.01, 0.06	0.01, 0.06	0.02, 0.05	0.01, 0.05	

p Values are based on the ranks of the biologic variables.

[a] Subset of participants with hemoglobin <200 ng/mL at all time points; excludes those missing hemoglobin values at one or more time points.

Table 4 Correlation between change in CSF biomarkers and change in the Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III motor score and the total score in patients with Parkinson disease (PD) and healthy controls (HC)

	Patients with PD				HC	
	Change at 6 months		Change at 12 months		Change at 12 months	
	Spearman correlation coefficient	*p* Value	Spearman correlation coefficient	*p* Value	Spearman correlation coefficient	*p* Value
Correlation with Aβ42						
MDS-UPDRS part III score	−0.08	0.296	0.01	0.879	−0.11	0.266
MDS-UPDRS total score	−0.13	0.082	−0.01	0.895	−0.01	0.916
Correlation with t-tau						
MDS-UPDRS part III score	−0.02	0.772	0.03	0.713	0.09	0.369
MDS-UPDRS total score	−0.07	0.369	−0.03	0.718	0.09	0.347
Correlation with p-tau						
MDS-UPDRS part III score	0.11	0.162	0.05	0.563	−0.00	0.999
MDS-UPDRS total score	0.09	0.247	0.06	0.471	−0.01	0.898
Correlation with α-syn						
MDS-UPDRS part III score	0.12	0.117	0.12	0.118	0.13	0.168
MDS-UPDRS total score	0.15	0.047	0.17	0.029	0.07	0.470
Correlation with α-syn[a] (low Hgb)						
MDS-UPDRS part III score	0.17	0.120	−0.02	0.882	0.10	0.515
MDS-UPDRS total score	0.19	0.097	−0.03	0.808	−0.12	0.460
Correlation with p-tau/t-tau						
MDS-UPDRS part III score	0.09	0.252	0.01	0.944	−0.04	0.650
MDS-UPDRS total score	0.08	0.304	0.04	0.587	−0.06	0.513
Correlation with t-tau/Aβ42						
MDS-UPDRS part III score	0.08	0.304	−0.03	0.737	0.19	0.047
MDS-UPDRS total score	0.04	0.611	−0.06	0.466	0.15	0.110
Correlation with p-tau/Aβ42						
MDS-UPDRS part III score	0.13	0.090	0.034	0.667	0.03	0.782
MDS-UPDRS total score	0.1291	0.090	0.056	0.475	−0.03	0.813
Correlation with t-tau/α-syn						
MDS-UPDRS part III score	−0.06	0.446	−0.06	0.475	−0.09	0.369
MDS-UPDRS total score	−0.11	0.147	−0.15	0.055	−0.07	0.480
Correlation with t-tau/α-syn[a]						
MDS-UPDRS part III score	−0.034	0.756	0.22	0.050	−0.04	0.787
MDS-UPDRS total score	−0.05	0.643	0.22	0.051	0.01	0.937

Abbreviations: α-syn = α-synuclein; Aβ42 = β-amyloid 1-42; p-tau = phosphorylated tau protein; Hgb = hemoglobin; t-tau = total tau protein.
Controls did not complete MDS-UPDRS at 6 months.
[a] Subset of participants with Hgb <200 ng/mL at all time points; excludes those missing Hgb values at one or more time points.

with PD at enrollment in the Deprenyl and Tocopherol Antioxidative Therapy of PD (DATATOP) placebo-controlled clinical trial revealed a slight but significant positive correlation between the rate of change in t-tau or t-tau/Aβ42 levels and changes in Unified Parkinson's Disease Rating Scale scores.[13] In the PPMI cohort, the correlation between clinical progression by total MDS-UPDRS scores and changes of CSF α-syn after 6 and 12 months of observation supports a pathophysiologic connection of CSF α-syn levels with motor progression, albeit weak. However, this is not directly related to measures of presynaptic dopamine integrity in the basal ganglia by DaTscan.

Since the PD phenotype is very heterogeneous, different subtypes could show different biomarker

Table 5 Longitudinal relationship between CSF biomarkers and Parkinson disease (PD) medications in patients with PD (calculated as levodopa equivalent dosages [LED] as published[19])

	Patients with PD	
Variable	**Estimate (95% CI)**	***p* Value**
Relationship with Aβ42		
PD medication use	−4.07 (−29.38 to 21.24)	0.750
Total LED	0.08 (−0.01 to 0.16)	0.074
LED subtotal—dopamine replacement	0.08 (−0.02 to 0.18)	0.114
LED subtotal—dopamine agonists	−0.00 (−0.18 to 0.18)	0.969
Relationship with t-tau		
PD medication use	5.76 (−8.81 to 20.33)	0.434
Total LED	0.03 (−0.01 to 0.08)	0.164
LED subtotal—dopamine replacement	0.03 (−0.03 to 0.09)	0.315
LED subtotal—dopamine agonists	0.04 (−0.06 to 0.14)	0.400
Relationship with p-tau		
PD medication use	−12.31 (−49.38 to 24.76)	0.510
Total LED	0.06 (−0.07 to 0.18)	0.365
LED subtotal—dopamine replacement	0.02 (−0.13 to 0.17)	0.808
LED subtotal—dopamine agonists	0.13 (−0.14 to 0.39)	0.340
Relationship with α-syn		
PD medication use	−28.54 (−48.40 to −8.69)	0.005
Total LED	−0.06 (−0.12 to 0.01)	0.073
LED subtotal—dopamine replacement	0.02 (−0.06 to 0.10)	0.560
LED subtotal—dopamine agonists	−0.28 (−0.41 to −0.14)	<0.0001
Relationship with α-syn[a]		
PD medication use	−43.24 (−71.34 to −15.15)	0.004
Total LED	−0.09 (−0.19 to 0.01)	0.077
LED subtotal—dopamine replacement	0.07 (−0.05 to 0.19)	0.260
LED subtotal—dopamine agonists	−0.42 (−0.59 to −0.25)	<0.0001
Relationship with p-tau/t-tau		
PD medication use	−7.10 (−45.86 to 31.65)	0.716
Total LED	0.05 (−0.08 to 0.18)	0.407
LED subtotal—dopamine replacement	−0.01 (−0.17 to 0.14)	0.878
LED subtotal—dopamine agonists	0.13 (−0.15 to 0.40)	0.373
Relationship with t-tau/Aβ42		
PD medication use	9.59 (−17.64 to 36.81)	0.485
Total LED	−0.01 (−0.10 to 0.08)	0.803
LED subtotal—dopamine replacement	−0.01 (−0.12 to 0.10)	0.862
LED subtotal—dopamine agonists	0.04 (−0.15 to 0.23)	0.682
Relationship with p-tau/Aβ42		
PD medication use	−11.09 (−47.81 to 25.62)	0.549
Total LED	0.04 (−0.09 to 0.16)	0.565
LED subtotal—dopamine replacement	−0.01 (−0.16 to 0.14)	0.891
LED subtotal—dopamine agonists	0.17 (−0.09 to 0.42)	0.212
Relationship with t-tau/α-syn		
PD medication use	39.36 (8.42 to 70.29)	0.013

Continued

dynamics. We found an increased t-tau/Aβ42 ratio in participants with RBD, which may be part of a clinical subtype of PD with faster progression[28] and has been associated with greater synuclein pathology in PD.[29] Other clinical subtypes need to be determined upon longer clinical follow-up.

Levels of Aβ42 and p-tau showed a small mean increase over 12 months in PD, and this was not associated with the *APOE* ε4 allele or with cognition (other than visuospatial deficits). Levels of CSF Aβ42 were generally above the cutoff developed in the Alzheimer's Disease Neuroimaging Initiative study (quantified by the same assay) of 192 pg/mL that has been validated against autopsy and against amyloid PET imaging.[30] Given the relatively young age (mean age 60.9 years) and early, de novo status of the PPMI PD cohort, it is likely that most participants do not yet have important coexisting amyloid plaque pathology. Among participants in PPMI, those with lower CSF Aβ42 at baseline have been shown to have greater risk of cognitive decline over 2 years.[31]

The potentially confounding effect of pharmacotherapy on biomarkers, especially α-syn, has not been investigated intensively. In PPMI, the first lumbar puncture was performed with all patients drug-naive, but with progressing motor symptoms, participants started dopaminergic treatment. The question of an effect of dopaminergic treatment on biomarker measurement has been raised previously[32] as D1, D2, D4, and D5 receptors are expressed in the choroid plexus, which, upon activation, could alter CSF homeostasis.[33] Some dopamine agonists decrease α-syn phosphorylation[34] and may protect against neuroinflammation[35] and may thus have neuroprotective properties. In fact, we found that patients using PD medications had greater changes in CSF α-syn, especially those on dopamine agonists. Whether the effect on CSF α-syn changes reflects these interactions and whether there are different binding properties of dopamine agonists will require further study.

Our focus was on 4 known core markers of neurodegeneration relevant to PD (i.e., α-syn, Aβ42, t-tau, and p-tau) with well-validated assays for their quantification in CSF. Similar to AD, it is highly likely that a panel of multiple biomarkers will be helpful to mirror the complex process of progressive neurodegeneration in PD. Additional PD biomarker candidates have been proposed based mainly on cross-sectional studies and may be candidates for longitudinal analysis; for example, phosphorylated[22] and oligomeric α-syn,[23,36] neurofilament light chains,[11] and others. In addition, there is a need to identify new biomarker candidates that may predict or track clinical progression in PD, either hypothesis-driven by the increasing knowledge of the pathologic

Table 5 Continued

	Patients with PD	
Variable	**Estimate (95% CI)**	***p* Value**
Total LED	0.10 (−0.00 to 0.20)	0.061
LED subtotal—dopamine replacement	−0.00 (−0.13 to 0.12)	0.991
LED subtotal—dopamine agonists	0.36 (0.14 to 0.58)	0.001
Relationship with t-tau/α-syn[a]		
PD medication use	68.10 (24.76 to 111.44)	0.003
Total LED	0.16 (0.01 to 0.32)	0.044
LED subtotal—dopamine replacement	−0.08 (−0.27 to 0.11)	0.400
LED subtotal—dopamine agonists	0.67 (0.41 to 0.94)	<0.0001

Abbreviations: α-syn = α-synuclein; Aβ42 = β-amyloid; CI = confidence interval; p-tau = phosphorylated tau; t-tau = total tau.
p Values are based on the ranks of the biologic variables.
[a] Subset of participants with hemoglobin <200 ng/mL at all time points; excludes those missing hemoglobin values at one or more time points.

processes or unbiased with continuously improving sensitive (e.g., -omics) technology. Although we did not find evidence for significant progression of the CSF biomarkers we studied during 12 months early in the course of PD, longer follow-up and expansion of these and other CSF biomarker panels in the PPMI and other cohorts will help to define a more detailed picture of biochemical events in the brain along the course of PD.

AUTHOR CONTRIBUTIONS

B.M., D.G., and C.S.C. designed the study and were responsible for data processing. C.J.C.-G. and C.S.C. oversaw all statistical analyses. P.T., L.M.S., J.Q.T., and A.S. were involved in sample analyses and data interpretation. M.F. and K.M. oversaw patient recruitment and assisted in the interpretation of data. B.M., C.J.C.-G., and D.G. wrote the manuscript. C.S.C., P.T., L.M.S., J.Q.T., A.S., M.F., and K.M. coedited the manuscript. B.M., D.G., and K.M. had full access to the clinical primary data. All authors had access to the data generated in the study including the statistical analysis and decided to submit the paper for publication.

ACKNOWLEDGMENT

The authors thank the Michael J. Fox Foundation, their PPMI colleagues, and the individuals who participated in this study.

STUDY FUNDING

PPMI is sponsored by the Michael J. Fox Foundation for Parkinson's Research (MJFF) and is cofunded by MJFF, AbbVie, Avid Radiopharmaceuticals, Biogen Idec, Bristol-Myers Squibb, Covance, Eli Lilly & Co., F. Hoffman-La Roche, Ltd., GE Healthcare, Genentech, GlaxoSmithKline, Lundbeck, Merck, MesoScale, Piramal, Pfizer, and UCB. This study is funded by The MJFF and funding partners, including Abbott, Biogen Idec, F. Hoffman-La Roche Ltd., GE Healthcare, Genentech, and Pfizer Inc. J.Q.T. is supported in part by P50 NS053488.

DISCLOSURE

B. Mollenhauer has received independent research grants from TEVA-Pharma, Desitin, Boehringer Ingelheim, and GE Healthcare; honoraria for consultancy from Bayer Schering Pharma AG, Roche, AbbVie, TEVA-Pharma, and Biogen, and for presentations from GlaxoSmithKline, Orion Pharma, and TEVA-Pharma; and travel costs from TEVA-Pharma. B.M. is a member of the executive steering committee of the Parkinson's Progression Marker Initiative and the Systemic Synuclein Sampling Study of the Michael J. Fox Foundation for Parkinson's Research (MJFF); has received grants from the BMBF, EU, Parkinson Fonds Deutschland, Deutsche Parkinson Vereinigung, MJFF, and Stifterverband für die deutsche Wissenschaft; and has scientific collaborations with Roche, Bristol Myers Squibb, Ely Lilly, Covance, and Biogen. C. Caspell-Garcia served as a consultant receiving fees from MJFF; and received research funding from The Michael J. Fox Foundation for Parkinson's Research. C. Coffey served as a consultant receiving fees from MJFF; and received research funding from NINDS, NHLBI, and MJFF. P. Taylor is an employee of BioLegend. L. Shaw receives funding from the NIH/NIA, U19 AG024904; from MJFF, Eli Lilly, and Roche; honoraria for consultancy from Novartis and Eli Lilly; and travel expenses from Eli Lilly and Roche and honorarium from Eli Lilly and Novartis; and has provided QC oversight for immunoassays produced by Fujirebio and Roche as part of the ADNI and PPMI studies. J. Trojanowski may accrue revenue in the future on patents submitted by the University of Pennsylvania wherein he is coinventor and received revenue from the sale of Avid to Eli Lily as coinventor on imaging-related patents submitted by the University of Pennsylvania; and receives research support from the NIH, GSK, Janssen, Biogen, and several nonprofits. A. Singleton was supported by grant Z01AG000949-06 from the Intramural Research Program, National Institute on Aging, NIH. Ju-Hee was supported by grant MRC 2014009392 from the National Research Foundation of Korea, Ministry of Science, ICT, and Future Planning. M. Frasier is an employee of MJFF. K. Marek is a consultant for Pfizer, GE Healthcare, Merck, Lilly, BMS, Piramal, Prothena, Neurophage, nLife, and Roche; receives funding for the following grants: W81XWH-06-1-0678, Establishing an 'at risk' cohort for Parkinson Disease Neuroprevention using olfactory testing and DAT imaging, DOD, Investigator 10/1/06–09/30/15; Parkinson's Progression Marker Initiative, Michael J. Fox Foundation, Principal Investigator 6/15/09–6/14/18; DAT imaging in LRRK2 family members, the Michael J. Fox Foundation, Principal Investigator 1/15/10–1/14/15; and has ownership in Molecular Neuro-Imaging, LLC. D. Galasko is supported by NIH grant AGO5131 and by the Michael J. Fox Foundation; and has provided consultation for vTv Pharmaceuticals, Eli Lilly, Inc., and Proclara, Inc. Go to Neurology.org for full disclosures.

Received February 12, 2017. Accepted in final form August 24, 2017.

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